

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



AR

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁴ : C07K 7/06, 7/26, A61K 37/43	A1	(11) International Publication Number: WO 88/ 05052 (43) International Publication Date: 14 July 1988 (14.07.88)
(21) International Application Number: PCT/US88/00051 (22) International Filing Date: 7 January 1988 (07.01.88) (31) Priority Application Number: 001,245 (32) Priority Date: 7 January 1987 (07.01.87) (33) Priority Country: US (71) Applicant: THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND [US/US]; 1430 Tulane Avenue, New Orleans, LA 70112 (US). (72) Inventors: COY, David, H. ; 4319 Perrier Street, New Orleans, LA 70115 (US). MURPHY, William, A. ; Route 8, Box 979, Covington, LA 70433 (US). (74) Agent: CLARK, Paul, T.; Fish & Richardson, One Financial Center, Suite 2500, Boston, MA 02111 (US).		(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HEPTAPEPTIDE (57) Abstract Heptapeptide analogs of somatastatin which inhibit secretion of growth hormone.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	ML	Mali
AU	Australia	GA	Gabon	MR	Mauritania
BB	Barbados	GB	United Kingdom	MW	Malawi
BE	Belgium	HU	Hungary	NL	Netherlands
BG	Bulgaria	IT	Italy	NO	Norway
BJ	Benin	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland				

- 1 -

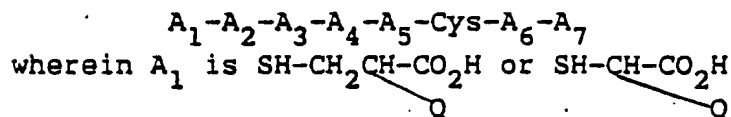
HEPTAPEPTIDEBackground of the Invention

This invention relates to therapeutic peptides.

A number of somatostatin analogs exhibiting
 5 GH-release-inhibiting activity have been described in
 the literature, including analogs containing fewer than
 the naturally occurring fourteen amino acids. For
 example, Coy et al. U.S. Patent No. 4,485,101, hereby
 incorporated by reference, describes dodecapeptides
 10 having an N-terminal acetyl group, a C-terminal NH₂,
 D-Trp at position 6, and p-Cl-Phe at position 4.
 (Herein, when no designation of configuration is given,
 the L-isomer is intended.)

Summary of the Invention

15 In general, the invention features a
 heptapeptide of the formula:



20 (where Q is H or a C₁-C₈ alkyl group); A₂ is o-,
 m-, or more preferably, p-substituted X-Phe or X-D-Phe
 (where X is H, halogen, NH₂, NO₂, OH, C₁-C₃
 alkyl, or C₁-C₃ alkoxy); A₃ is X-Trp, X-D-Trp,
 α-N-methyl-X-Trp, or α-N-methyl-D-X-Trp (where X is
 25 a substituent on the benzene ring and is H, halogen,
 NH₂, NO₂, OH, C₁-C₃ alkyl, or C₁-C₃ alkoxy);
 A₄ is Lys, α-N-methyl-Lys, or ε-N-R₁-Lys
 (where R₁ is C₁-C₃ alkyl); A₅ is Val

30 or Thr; A₆ is Pro or $-\overset{\text{O}}{\parallel}\text{C}-\text{N}-\text{CH}-\text{T}$, where Z is H or CH₃
 and T is H, CH₂OH, CH₂CH₂OH, CH₂CH₂CH₂OH,
 CH(CH₃)OH, isobutyl, benzyl (substituted in the o-,

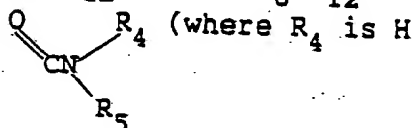
- 2 -

m-, or p- positions with H, halogen, NH_2 , NO_2 , OH, $\text{C}_1\text{-C}_3$ alkyl, or $\text{C}_1\text{-C}_3$ alkoxy), CH_2 - β -naphthyl (substituted on the benzene ring with H, halogen, NH_2 , OH, $\text{C}_1\text{-C}_3$ alkyl, or $\text{C}_1\text{-C}_3$ alkoxy), or

5 CH_2 -pyridyl (substituted on the benzene ring with H, halogen, NH_2 , NO_2 , OH, $\text{C}_1\text{-C}_3$ alkyl, or $\text{C}_1\text{-C}_3$

alkoxy); and A_7 is COR_2 (where R_2 is H or $\text{C}_1\text{-C}_3$ alkyl), CH_2OH , CH_2OCR_3 (where R_3 is $\text{C}_1\text{-C}_{12}$ alkyl, $\text{C}_8\text{-C}_{12}$ aralkyl, or phenoxy), or

10 $\text{C}_1\text{-C}_{12}$ alkyl, $\text{C}_8\text{-C}_{12}$ aralkyl, or phenoxy), or



or $\text{C}_1\text{-C}_3$ alkyl and R_5 is H, $\text{C}_1\text{-C}_3$ alkyl, phenyl, or $\text{C}_7\text{-C}_{10}$ aralkyl); or a pharmaceutically acceptable salt thereof.

15

In the formula given above, the configuration of the molecule at the carbon atom to which T is bonded is not given, indicating that the amino acid residue of which T is a substituent can have the D- or L- configuration.

20

Preferred compounds of the invention include MPA-Tyr-D-Trp-Lys-Val-Cys-Thr- NH_2 (MPA = mercaptopropionic acid);

25 MPA-Tyr-D-Trp-Lys-Val-Cys-Phe- NH_2 ; MPA-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe- NH_2 ; and MPA-Tyr-D-Trp-Lys-Val-Cys- β -Nal- NH_2 .

In other preferred embodiments, a therapeutically effective amount of the therapeutic compound and a pharmaceutically acceptable carrier substance, e.g. magnesium carbonate, lactose, or a phospholipid with which the therapeutic compound can form a micelle, together form a therapeutic composition, e.g. a pill, tablet, capsule, or liquid for oral

30

- 3 -

administration to a human patient, a spreadable cream, gel, lotion, or ointment for application to the skin of a human patient in need of the compound, a liquid capable of being administered nasally as drops or spray, or a liquid capable of intravenous, parenteral, subcutaneous, or intraperitoneal administration. The pill, tablet or capsule can be coated with a substance capable of protecting the composition from the gastric acid in the patient's stomach for a period of time sufficient to allow the composition to pass undisintegrated into the patient's small intestine. The therapeutic composition can also be in the form of a biodegradable sustained release formulation for intramuscular administration. For maximum efficacy, zero order release is desired. Zero order release can be obtained using an implantable or external pump, e.g., InfusoidTM pump, to administer the therapeutic composition.

The compounds of the invention are active in inhibiting the secretion of GH, insulin, and glucagon.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

25. Structure

The compounds of the invention have the general formula recited in the Summary of the Invention, above. They are all heptapeptide analogs of somatostatin which have Cys at position 6; this residue forms a ring with the residue at position 1 via a disulfide bond. It has been found that MPA at position 1; Tyr at position 2; D-Trp at position 3; Lys at position 4; and Val at position 5 are modifications which particularly enhance activity.

- 4 -

The compounds can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pantoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid.

Synthesis

The synthesis of one heptapeptide follows. Other heptapeptides can be prepared by making appropriate modifications, within the ability of someone of ordinary skill in this field, of the following synthetic method.

The first step in the preparation of MPA-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂ was the preparation of the intermediate S-methylbenzyl-3-MPA-Tyr-D-Trp-N-benzyloxycarbonyl-Lys-Val-S-methylbenzyl-Cys-O-benzyl-Thr-benzhydryl amine resin, as follows.

Benzhydrylamine-polystyrene resin (Vega Biochemicals, Inc.) (1.00 g, 0.5 mmole) in the chloride ion form was placed in the reaction vessel of a Beckman 990B peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 25 min. each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% triethylamine in chloroform.

- 5 -

The neutralized resin was stirred with Boc-O-benzyl- threonine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 h; the resulting amino acid resin was then cycled through steps (a) to (g) in the above wash program. Next, the following amino acids (1.5 mmole) were coupled successively by the same procedure: Boc-S-methylbenzyl-cysteine, Boc-Val, Boc-N-benzyloxycarbonyl- lysine, Boc-D-Trp, Boc-tyrosine, and Boc-S-methylbenzyl-3-mercaptopropionic acid. After washing and drying, the completed resin weighed 1.89 g.

The resin was mixed with anisole (4 ml) and anhydrous hydrogen fluoride (36 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride was evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide was then dissolved in 800 ml of 90% acetic acid to which was added I₂ in methanol until a permanent brown color was present. The solution was then stirred for 1 h before removing the solvent in vacuo. The resulting oil was dissolved in a minimum volume of 50% acetic acid and eluted on a column (2.5 X 100 mm) of Sephadex G-25. Fractions containing a major component by uv absorption and thin layer chromatography were then pooled, evaporated to a small volume, and applied to a column (2.5 X 50 cm) of Whatman LRP-1 octadecylsilane (15-20 μ M).

The column was eluted with a linear gradient of 10-50% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions were examined by thin layer chromatography and analytical high performance liquid chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water yielded 129 mg of the product as a white, fluffy powder.

- 6 -

The product was found to be homogeneous by Hplc and Tlc. Amino acid analysis of an acid hydrolysate and fast atom bombardment mass spectrometry confirmed the composition of the heptapeptide.

5 The heptapeptides of the invention having the formulae MPA-Tyr-D-Trp-Lys-Val-Cys-Phe-NH₂; MPA-Tyr-D-Trp-Lys-Val-Cys- p-Cl-Phe-NH₂; and MPA-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH₂ were made according to methods analogous to those described above.

10 Use

When administered to mammals, particularly humans, (e.g. orally, topically, intravenously, parenterally in a sustained release, biodegradable form, nasally, or by suppository), the compounds can be effective to inhibit GH release as well as to inhibit insulin, glucagon, and pancreatic exocrine secretion, and to therapeutically affect the central nervous system.

15 The compounds can be administered to a mammal, e.g. a human, in the dosages used for somatostatin or, because of their greater potency, in smaller dosages. The compounds of the invention can be used for the treatment of cancer, particularly growth hormone-dependent cancer (e.g., bone, cartilage, pancreas (endocrine and exocrine), prostate, or breast), acromegaly and related hypersecretory endocrine states, or of bleeding ulcers in emergency patients and in those suffering from pancreatitis or diarrhea. The compounds can also be used in the management of diabetes and to protect the liver of patients suffering from cirrhosis and hepatitis. The compounds can also be used to treat Alzheimer's disease, as analgesics to treat pain by acting specifically on certain opiate receptors, and as

- 7 -

gastric cytoprotective compounds for ulcer therapy. The compounds can also be used to treat certain types of mushroom poisoning.

5 The compounds can also be used to treat diabetes-related retinopathy. The anti-cancer activity of the compounds may be related to their ability to antagonize cancer-related growth factors such as epidermal growth factor.

10 The compounds can be administered to a mammal, e.g., a human, in a dosage of 0.01 to 1000 mcg/kg/day, preferably 0.1 to 100 mcg/kg/day.

Other embodiments are within the following claims.

- 9 -

alkyl and R_5 is H, C_1-C_3 alkyl, phenyl, or C_7-C_{10} aralkyl); or a pharmaceutically acceptable salt thereof.

2. The heptapeptide of claim 1 having the
5 formula methylpropionic
acid-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂, or a
pharmaceutically acceptable salt thereof.

3. The heptapeptide of claim 1 having the
formula methylpropionic
10 acid-Tyr-D-Trp-Lys-Val-Cys-Phe-NH₂, or a
pharmaceutically acceptable salt thereof.

4. The heptapeptide of claim 1 having the
formula methylpropionic
acid-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe-NH₂, or a
15 pharmaceutically acceptable salt thereof.

5. The heptapeptide of claim 1 having the
formula methylpropionic
acid-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH₂, or a
pharmaceutically acceptable salt thereof.

20 6. A therapeutic composition capable of
inhibiting the release of growth hormone, insulin,
glucagon, or pancreatic exocrine secretion comprising a
therapeutically effective amount of the compound of
claim 1 together with a pharmaceutically acceptable
25 carrier substance.

7. A method of treating a mammal in need of
reduction of growth hormone, insulin, glucagon, or
pancreatic exocrine secretion comprising administering
to said mammal a therapeutically effective amount of the
30 compound of claim 1.

- 10 -

8. The therapeutic composition of claim 6 wherein said composition is in the form of a pill, tablet, or capsule for oral administration to a human patient in need of said compound.

5 9. The therapeutic composition of claim 6 wherein said composition is in the form of a liquid for oral administration to a human patient in need of said compound.

10 10. The therapeutic composition of claim 8, said composition being coated with a substance capable of protecting said composition from the gastric acid in the stomach of said human patient for a period of time sufficient to allow said composition to pass undisintegrated into the small intestine of said human
15 patient.

11. The therapeutic composition of claim 6, said composition being in the form of a cream, gel, spray, or ointment for application to the skin of a human patient in need of said compound.

20 12. The therapeutic composition of claim 6, said composition being in the form of a liquid capable of being administered nasally as drops or spray to a human patient in need of said compound.

25 13. The therapeutic composition of claim 6, said composition being in the form of a liquid for intravenous, subcutaneous, parenteral, or intraperitoneal administration to a human patient in need of said compound.

30 14. The therapeutic composition of claim 6, said composition being in the form of a biodegradable sustained release composition for intramuscular administration to a human patient in need of said compound.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/00051

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC(4): C07K 7/06, 7/26; A61K 37/43 U.S. CL: 530/311, 530/329; 514/16																													
II. FIELDS SEARCHED <div style="text-align: center; margin-top: 5px;">Minimum Documentation Searched ⁷</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 20%;">Classification System</th> <th style="width: 80%;">Classification Symbols</th> </tr> <tr> <td style="text-align: center; padding: 5px;">U.S.</td> <td style="padding: 5px;">530/311, 530/329; 514/16</td> </tr> </table> <div style="text-align: center; margin-top: 5px;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	U.S.	530/311, 530/329; 514/16																							
Classification System	Classification Symbols																												
U.S.	530/311, 530/329; 514/16																												
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%;">Category [*]</th> <th style="width: 60%;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 30%;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,190,648 (VEBER), 26 February 1980. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,291,022 (SANDRIN ET AL), 22 September 1981. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,395,403 (BAUER ET AL), 26 July 1983. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,485,101 (COY ET AL), 27 November 1984. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,435,385 (BAUER ET AL), 06 March 1984. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,486,415 (FREIDINGER), 04 December 1984. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,522,813 (NUTT), 11 June 1985. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">A</td> <td style="vertical-align: top;">US, A, 4,585,755 (MORGAN ET AL), 29 April 1986. See entire document.</td> <td style="text-align: center; vertical-align: top;">1-14</td> </tr> </table>			Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	A	US, A, 4,190,648 (VEBER), 26 February 1980. See entire document.	1-14	A	US, A, 4,291,022 (SANDRIN ET AL), 22 September 1981. See entire document.	1-14	A	US, A, 4,395,403 (BAUER ET AL), 26 July 1983. See entire document.	1-14	A	US, A, 4,485,101 (COY ET AL), 27 November 1984. See entire document.	1-14	A	US, A, 4,435,385 (BAUER ET AL), 06 March 1984. See entire document.	1-14	A	US, A, 4,486,415 (FREIDINGER), 04 December 1984. See entire document.	1-14	A	US, A, 4,522,813 (NUTT), 11 June 1985. See entire document.	1-14	A	US, A, 4,585,755 (MORGAN ET AL), 29 April 1986. See entire document.	1-14
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³																											
A	US, A, 4,190,648 (VEBER), 26 February 1980. See entire document.	1-14																											
A	US, A, 4,291,022 (SANDRIN ET AL), 22 September 1981. See entire document.	1-14																											
A	US, A, 4,395,403 (BAUER ET AL), 26 July 1983. See entire document.	1-14																											
A	US, A, 4,485,101 (COY ET AL), 27 November 1984. See entire document.	1-14																											
A	US, A, 4,435,385 (BAUER ET AL), 06 March 1984. See entire document.	1-14																											
A	US, A, 4,486,415 (FREIDINGER), 04 December 1984. See entire document.	1-14																											
A	US, A, 4,522,813 (NUTT), 11 June 1985. See entire document.	1-14																											
A	US, A, 4,585,755 (MORGAN ET AL), 29 April 1986. See entire document.	1-14																											
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>																													
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search 14 April 1988 </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report 24 MAY 1988 </td> </tr> <tr> <td style="width: 50%; padding: 5px;"> International Searching Authority ISA/US </td> <td style="width: 50%; padding: 5px;"> Signature of Authorized Officer <i>Christina Chan</i> Christina Chan </td> </tr> </table>			Date of the Actual Completion of the International Search 14 April 1988	Date of Mailing of this International Search Report 24 MAY 1988	International Searching Authority ISA/US	Signature of Authorized Officer <i>Christina Chan</i> Christina Chan																							
Date of the Actual Completion of the International Search 14 April 1988	Date of Mailing of this International Search Report 24 MAY 1988																												
International Searching Authority ISA/US	Signature of Authorized Officer <i>Christina Chan</i> Christina Chan																												

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

- | | | |
|---|---|------|
| A | <p><u>Life Sciences</u>, (New York, USA), Volume 34, issued 1984, (VEBER ET AL), "A super active cyclic hexapeptide analog of somatostatin". See pages 1371-1378.</p> | 1-14 |
|---|---|------|

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter^{1,2} not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out^{1,2}, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.